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Correlation of Interferon Treatment Response With GBV-C/HGV Genomic RNA and Anti-Envelope 2 Protein Antibody

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The clinical significance of GB virus C/hepatitis G virus (GBV-C/HGV) co-infection was studied retrospectively in 100 consecutive patients with hepatitis C virus (HCV) infection. All 100 patients had been treated with interferon-α (IFN-α). Co-infection with GBV-C/HGV and HCV was detected in 10 of the 100 patients (10%) and anti-envelope 2 region (anti-E2) antibody was detected in 25 patients. None of the patients with GBV-C/HGV RNA had anti-E2 antibody. Co-infected patients were younger ($P < .005$) and their serum transaminase levels were lower than HCV-only infected patients ($P < .01$). In 7 of the 10 co-infected patients, HCV RNA was eradicated from serum after IFN-α treatment and normal alanine transaminase (ALT) levels continued in 6 of these 7 patients. In one patient who was negative for HCV RNA but positive for GBV-C/HGV RNA, the ALT level relapsed transiently. The rate of clearance of HCV and normalization of the ALT level was significantly higher in co-infected patients than in HCV-only infected patients ($P < .05$). GBV-C/HGV RNA disappeared from 6 of the 10 co-infected patients (60%) upon cessation of IFN-α treatment. However, continuous clearance of GBV-C/HGV was observed in only two patients and anti-E2 antibody could not be detected in the serum of these patients. These results indicate that co-infected patients tend to be younger and more sensitive to IFN-α treatment. However, long-term clearance of GBV-C/HGV after IFN-α treatment may be difficult. Moreover, anti-E2 antibody may act to neutralize GBV-C/HGV. ***J. Med. Virol. 57:370–375, 1999.***

KEY WORDS: GBV-C/HGV; hepatitis C virus; co-infection; anti-E2 antibody; interferon

INTRODUCTION

Recently, two new hepatitis viruses have been cloned and characterized, namely GB virus C (GBV-C) [Simons et al., 1995] and hepatitis G virus (HGV) [Linnen et al., 1996]. The nucleotide and amino acid sequences of these two viruses are highly homologous [Zuckerman, 1996], and thus these viruses are believed to be different isolates of the same virus. GBV-C/HGV is a flavivirus with an approximately 10-kb long, single-stranded RNA genome of positive polarity. GBV-C/HGV infection is usually diagnosed by the detection of genomic RNA using the reverse-transcription polymerase chain reaction (RT-PCR).

Although recent studies have suggested that GBV-C/HGV may cause fulminant hepatitis [Yoshiba et al., 1995; Heringlake et al., 1996], the clinical significance of GBV-C/HGV remains unclear. In addition, it is unclear whether persistent infection with GBV-C/HGV causes severe hepatic damage and progresses to chronic hepatitis, liver cirrhosis, or hepatocellular carcinoma. Moreover, GBV-C/HGV co-infection with hepatitis C virus (HCV) is common. Saiz et al. [1997] reported that GBV-C/HGV co-infection did not modify either the clinical characteristics or the response to interferon (IFN) in HCV carriers. However, the clinical significance of co-infection with HCV and GBV-C/HGV remains unclear. Recent studies have identified the importance of both HCV genotype [Kanai et al., 1992; Yoshioka et al., 1992; Kohara et al., 1995] and pretreatment serum levels of HCV RNA for predicting response to IFN-α therapy [Lau et al., 1993]. However, the rate

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of virological response to IFN-α in patients co-infected with GBV-C/HGV and HCV has yet to be clarified.

Recently, a new assay for an antibody to the GBV-C/HGV envelope 2 region was developed by Tacke et al. [1997]. Anti-E2 is thought to be a neutralizing antibody. The aim of the present study was to determine the relationships among co-infection with GBV-C/HGV and HCV, anti-E2 antibody, pretreatment HCV RNA levels, HCV genotypes, and response to IFN-α therapy in 100 consecutive patients with HCV infection.

MATERIALS AND METHODS

Patients

One hundred consecutive chronic hepatitis patients (65 male, 35 female), treated with natural IFN-α in our hospital were examined retrospectively. Both anti-HCV second-generation antibodies and HCV RNA were detected in their sera. Alanine transaminase (ALT) levels were abnormal for more than 6 months before IFN treatment. These patients had no history of treatment with immunomodulatory agents and had neither HBs antigen nor human immunodeficiency virus infection. Liver biopsy specimens were obtained from all patients for histological evaluation.

IFN Treatment Schedule and Response to IFN

Six megaunits of natural IFN-α (Sumiferon, Sumitomo Pharm. Co., Osaka, Japan) were administered every day for 2 weeks and then three times weekly for 22 weeks. Response to IFN-α treatment was evaluated biochemically using conventional criteria. Briefly, complete response (CR) to IFN-α was defined as normalization of serum ALT levels at the end of therapy. Sustained response (CR-Sus) was defined as maintenance of normal ALT levels throughout the follow-up period (at least 6 months) and indicated patients from whose serum HCV RNA disappeared for more than 12 months after IFN therapy. Some patients showed CR, but then showed increased ALT levels (biochemical relapse) shortly after the cessation of IFN-α treatment. These patients were designated CR-Rel. In addition, in CR-Rel patients, HCV RNA disappeared during IFN-α treatment but reappeared after cessation of IFN-α treatment. Patients not showing CR to IFN-α therapy were defined as nonresponders (NR).

Detection and Quantification of HCV RNA

Serum HCV RNA levels were quantified by RT-PCR. All serum samples were stored immediately at −80°C after collection. Total RNA was extracted from 0.1 ml of serum by guanidium thiocyanate, followed by phenol and phenol/chloroform extraction. Complementary DNA was synthesized with 200 U of Moloney murine leukemia virus reverse transcriptase (GIBCO-BRL, Grand Island, NY) and 50 pM of first-round PCR primers. PCR was conducted using primers derived from the highly conserved 5′ untranslated region (UTR), as described previously [Kohara et al., 1995]. HCV RNA was quantified by competitive RT-PCR. Thirty-five amplification cycles were carried out for both the first and second rounds of PCR. PCR products were analyzed by electrophoresis using 3% NuSieve 3:1 agarose (FMC Corporation, BioProducts Division, Rockland, ME). Each sample was analyzed at least twice to ensure reproducibility.

Serological Genotyping of HCV

The HCV genotype was determined using an HCV genotype-specific antibody assay described previously [Tanaka et al., 1994]. cDNA clones encoding 85 amino acids (amino acids 1676–1760) of the NS4 region of HCV genotypes 1 and 2 were expressed in *Escherichia coli* (strain C600) as fusion proteins with a portion of TrpE. Purified peptides were used as genotype-specific enzyme-linked immunosorbent assay (ELISA) antigens for genotype 1 HCV (C14-1) and genotype 2 HCV (C14-2).

Detection of GBV-C/HGV RNA

GBV-C/HGV genomic RNA was extracted from 100 μl of serum. The presence and level of GBV-C/HGV RNA was determined using PCR with the 5′ noncoding region (5′ NCR) and NS5a region as primers. RT-PCR was conducted as described previously by Schlueter et al. [1996].

Detection of Anti-E2 Antibody

Recombinant GBV-C/HGV envelope protein was used as the antigen in an ELISA carried out according to the method of Tacke et al. [1997]. This antigen was expressed under a cytomegalovirus-promoter in Chinese hamster ovary (CHO) cells. The cells were lysed in phosphate-buffered saline within 0.5% Nonidet P40 detergent. The solubilized protein product was bound via the biotin-conjugated FLAG-specific antibody M1 (Kodak) onto a streptavidin-coated microtiter plate. Plates were incubated with diluted sample, and anti-E2 antibody from the serum that bound to the E2 antigen was detected using anti-human IgG-peroxidase conjugate, and ABTS as the peroxidase substrate. Extinction was measured at 405 nm.

Statistical Analysis

All data were analyzed using the Mann–Whitney rank test, Student's t-test and χ^2- test. Statistical significance was established at the $P < .05$ level.

RESULTS

Table I shows the prevalence rate of GBV-C/HGV and anti-E2 antibody in HCV carriers of each genotype. GBV-C/HGV RNA was detected in the sera of 10 of 100 patients (10%) infected with HCV. Anti-E2 antibody was detected in 25 of the 100 patients (25%). None of the patients with GBV-C/HGV RNA had anti-E2 antibody and GBV-C/HGV RNA was not detected in any of the patients with anti-E2 antibody. This result suggests that anti-E2 antibody may have a neutralizing effect on GBV-C/HGV, as reported previously by Pilot-

TABLE I. Prevalence of HGV RNA and Anti-E2 Antibody in HCV Carriers

HCV type	HGV-related: HGV RNA	HGV-related: Anti-E2 antibody	HGV-related: HGV RNA/ Anti-E2[b]	Negative for HGV	Total
Genotype 1	3	9	0	45	57
Genotype 2	5	13	0	17	35
Genotype 1 + 2[a]	1	3	0	0	4
ND	1	0	0	3	4
Total	10	25	0	65	100

HGV, hepatitis G virus; HCV, hepatitis C virus; ND, not determined.
[a]Co-infection with HCV genotype 1 and 2.
[b]Both HGV RNA and anti-E2 are positive.

TABLE II. Clinical Characteristics of IFN-Treated Patients

Characteristic	HCV/HGV[a]	HCV[b]	
N	10	90	
Age	41.5 ± 11.7	52.2 ± 10.0*	
Sex (M:F)	8:2	57:33	
BT (+/–)	4:6	46:44	
AST (IU/L)	53 ± 15	83 ± 50*	
ALT (IU/L)	87 ± 22	131 ± 88*	
γGTP (IU/L)	48 ± 33	67 ± 57	
Histology			
CAH mild	8	58	
CAH severe	2	32	
HCV Genotype			
1	3	54	
2	5	30	
1 + 2	1	3	
ND	1	3	
IFN Response			
CR-Sus	7	33	**
CR-Rel	2	34	
NR	1	23	

IFN, interferon; HCV, hepatitis C virus; HGV, hepatitis G virus; BT, blood transfusion; AST, aspartate transaminase; ALT, alanine transaminase; CAH, congenital adrenal hyperplasia; ND, not determined; CR, complete response; Sus, sustained; Rel, relapse; NR, nonresponse.
[a]HCV/HGV: co-infected cases.
[b]HCV: HCV-only infected cases.
*$P < .01$.
**$P < .05$.

TABLE III. Responsibility of IFN Treatment by Distinctions of Age

Age (years)	HCV/HGV: CR-Sus	HCV/HGV: CR-Rel	HCV/HGV: NR	HCV: CR-Sus	HCV: CR-Rel	HCV: NR	
<50	5	2	1	9	18	3	NS
50≤	2	0	0	24	16	20	
Total	7	2	1	33	34	23	

IFN, interferon; HCV, hepatitis C virus; HGV, hepatitis G virus; CR, complete response; Sus, sustained; Rel, relapse; NR, nonresponse; NS, not significant.

Matias et al. [1996] and Tacke et al. [1997]. Moreover, 6 of the 10 patients with GBV-C/HGV infection, including those co-infected with HCV genotypes 1 and 2, had genotype 2 HCV infection and 16 of the 25 patients with anti-E2 antibody had genotype 2 HCV. This result suggests that co-infection with GBV-C/HGV and genotype 2 HCV is more common than co-infection with genotype 1 HCV ($P < .05$).

The clinical characteristics of the 100 patients examined are shown in Table II. No statistically significant differences in sex and history of blood transfusion were detected between the co-infected and the HCV-only infected groups. However, the co-infected patients are significantly younger than HCV-only infected patients ($P < .01$). In addition, although the level of serum transaminases (aspartate and alanine transaminases [AST and ALT, respectively]) was significantly lower in co-infected patients ($P < .01$), no difference in the γGTP level was detected between the two groups. With respect to histological severity, no significant difference between co-infected patients and HCV-only infected patients was observed. Forty of the 100 patients (40%) were classified as CR-Sus, 36 as CR-Rel, and the remaining 24 as NR. Thirty-three of the 90 patients (37%) infected with HCV-only and 7 of the 10 patients (70%) co-infected with GBV-C/HGV and HCV, were classified as CR-Sus. The rate of CR-Sus was significantly higher in the co-infected group than in the HCV-only infected group ($P < .05$). This difference may be because co-infected patients have a high rate of genotype 2 HCV infection and HCV-only infected patients are infected primarily with genotype 1 HCV. Patients co-infected with GBV-C/HGV were younger than HCV-only infected patients, and so we examined whether the effect of age on IFN response. Table III shows IFN response according to age. We found no statistically significant difference in IFN response between the two age groups.

Figure 1 shows the clinical course of CR-Sus patients during and after IFN treatment in co-infected cases (Fig. 1A) and HCV-only infected cases (Fig. 1B). Six of the seven CR-Sus co-infected patients maintained normal ALT levels despite the presence of GBV-C/HGV. HCV RNA was no longer detected in the sera of these six patients. The serum ALT level relapsed transiently in one patient (case 3, Fig. 1A). As just noted, HCV was not detectable but GBV-C/HGV remained in the serum of this patient (case 3, Table 5). On the other hand, HCV RNA remained undetectable from the sera of patients infected with HCV only and their ALT levels have remained normal. These results suggest that GBV-C/HGV may cause hepatic injury and that patients co-infected with GBV-C/HGV and HCV genotype 2 have a high tendency to be CR-Sus.

Figure 2 and Table IV show the change in GBV-C/

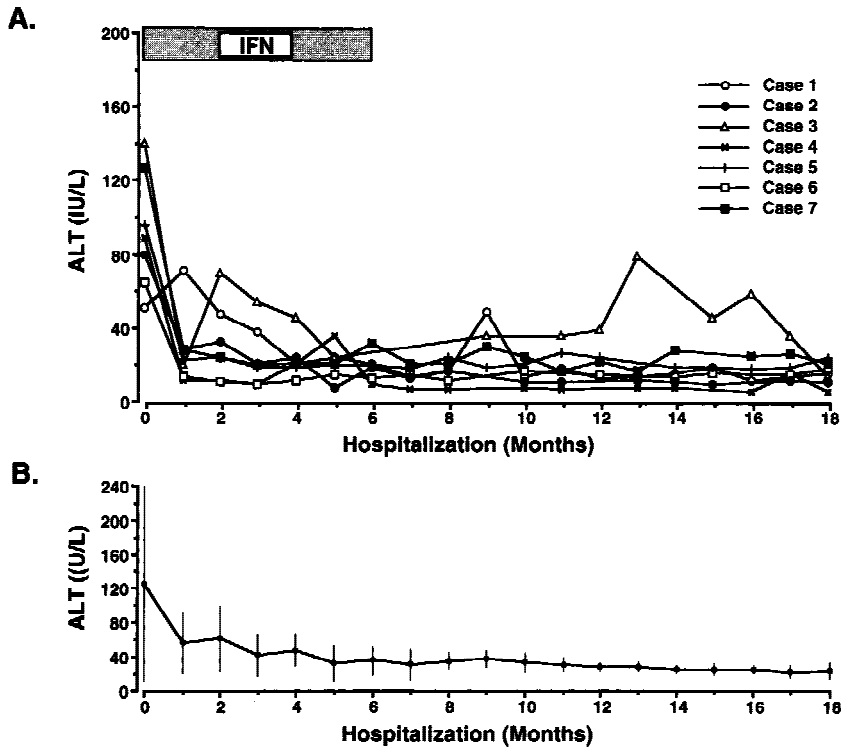

Fig. 1. **A:** Change in serum alanine transaminase (ALT) level of complete and sustained response patients with co-infection of GB virus C/hepatitis G virus (GBV-C/HGV) and hepatitis C virus (HCV). **B:** Change in serum ALT level (mean ± SD) in complete response and sustained response to interferon (IFN) of patients infected with HCV only.

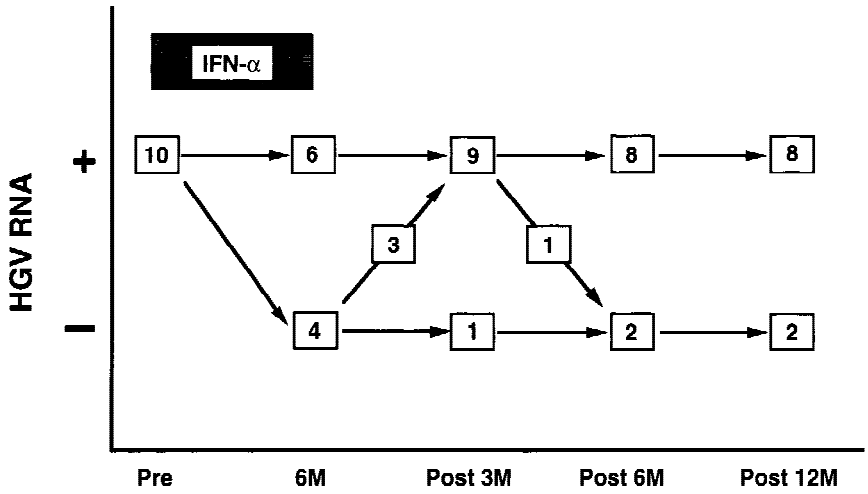

Fig. 2. Change in presence of GB virus C/hepatitis G virus (GBV-C/HGV) RNA during and after interferon-α (IFN-α) treatment in the 10 patients co-infected with GBV-C/HGV and hepatitis C virus.

HGV RNA in serum during and after IFN-α treatment. In 4 of the 10 patients co-infected with GBV-C/HGV and HCV, GBV-C/HGV RNA disappeared from the patients' sera during IFN-α treatment. However, GBV-C/HGV RNA reappeared after cessation of IFN-α treatment in two of these four patients.

Table V shows the HCV RNA copy number, anti-E2 antibody, HCV genotype, and existence of GBV-C/HGV RNA in the 10 co-infected patients. In patients in whom GBV-C/HGV was eradicated, anti-E2 did not appear during the 1-year follow-up period.

TABLE IV. Status of HCV and HGV In HCV and HGV Co-Infected Patients After IFN Treatment

	HGV RNA in Serum						
	Before IFN	Upon cessation of IFN		6 Months after IFN		12 Months after IFN	
HCV RNA in serum	+	+	−	+	−	+	−
+	10	1	0	2	1	2	1
−	0	5	4	6	1	6	1
Total	10	6	4	8	2	8	2

HCV, hepatitis C virus; HGV, hepatitis G virus; IFN, interferon.

TABLE V. HCV RNA Copy Number, Genotype and Existence of HGV RNA in the 10 Co-Infected Patients

			Before IFN treatment					1 Year after IFN treatment		
			HCV		HGV			HCV	HGV	
	Sex	Age (Years)	Genotype	HCV RNA (10^n copy/ml)	5′ UTR	NS 5	Anti-E2	HCV RNA	HGV RNA	Anti-E2
Case 1	F	30	1	7	(+)	(+)	(–)	(–)	(–)	(–)
Case 2	M	56	2	6	(+)	(+)	(–)	(–)	(+)	(–)
Case 3	M	49	2	5	(+)	(+)	(–)	(–)	(+)	(–)
Case 4	M	49	2	5	(+)	(+)	(–)	(–)	(+)	(–)
Case 5	M	35	2	5	(+)	(+)	(–)	(–)	(+)	(–)
Case 6	M	30	2	3	(+)	(+)	(–)	(–)	(+)	(–)
Case 7	M	24	1+2	4	(+)	(+)	(–)	(–)	(+)	(–)
Case 8	F	45	ND	7	(+)	(+)	(–)	(+)	(–)	(–)
Case 9	M	39	1	7	(+)	(+)	(–)	(+)	(+)	(–)
Case 10	M	58	1	6	(+)	(+)	(–)	(+)	(+)	(–)

HCV, hepatitis C virus; HGV, hepatitis G virus; IFN, interferon; ND, not determined.

DISCUSSION

We conducted a retrospective study of the clinical significance of co-infection with GBV-C/HGV and HCV in 100 Japanese patients treated with IFN-α for chronic hepatitis. The incidence of co-infection was 10%, which is about half of the HCV and GBV-C/HGV co-infection rate reported in the USA and Europe (about 20%) [Alter, 1996]. However, 25 of the 90 patients (28%) without GBV-C/HGV RNA were positive for anti-E2 antibody and these 25 patients may have had a past history of GBV-C/HGV infection. This result suggests that the total rate of co-infection with HCV may be 35%. An epidemiological study in Japan has revealed that 70–75% of HCV carriers have genotype 1 HCV infection and 20–25% have genotype 2 HCV [Tanaka et al., 1995]. However, in the present study, 50% (5/10) of HCV and GBV-C/HGV co-infected patients and 52% (13/25) of anti-E2 positive patients had genotype 2 HCV infection (Tables I and II), which suggests that GBV-C/HGV infection correlates with genotype 2 HCV infection ($P < .01$). The reason for frequent co-infection with GBV-C/HGV and genotype 2 HCV is unknown. Genotype 2 HCV infection may cause milder liver dysfunction than genotype 1 HCV infection and may not interfere in GBV-C/HGV replication.

The clinical features of the 10 co-infected patients were compared with those of the other 90 patients. Patients co-infected with GBV-C/HGV and HCV were younger and their transaminase levels were lower than patients infected with HCV only (Table II). However, no difference in histological progression was observed between the two groups. These results suggest that the duration of infection of GBV-C/HGV may be shorter than that of HCV. Whether GBV-C/HGV interferes with the replication of HCV is also unclear. Co-infection of HCV carriers with GBV-C/HGV does not increase and may decrease HCV-induced hepatocyte damage. Some researchers [Lau et al., 1997; Saiz et al., 1997] have reported that GBV-C/HGV co-infection of HCV-positive chronic hepatitis patients did not modify the clinical features and IFN response to HCV. This result differs from ours for unknown reasons, but may be related to genotypic differences between Japanese and European GBV-C/HGV. On the other hand, Franscesconi et al. [1997] reported recently that HCV carriers co-infected with GBV-C/HGV are younger and respond to IFN treatment better than HCV-only infected patients.

Many patients worldwide with chronic hepatitis C are treated and the response of HCV infection to IFN-α has been investigated by many researchers [Davis et al., 1989; Di Bisceglie et al., 1989]. In Japan, about 30–40% of chronic hepatitis C infection respond to IFN-α treatment. In these patients, HCV RNA disappears from the serum and serum ALT remains within the normal range on a long-term basis [Kanai et al., 1992; Yoshioka et al., 1992; Kohara et al., 1995]. HCV viral load and HCV genotype are important predictors of the response to IFN-α. That is, low viral load and genotype 2 HCV are more sensitive to IFN-α than a high viral load and genotype 1 HCV [Kohara et al., 1995]. In the present study, 5 of the 10 patients co-infected with GBV-C/HGV and HCV had genotype 2 HCV infection. In addition, the co-infected patients were more sensitive than HCV-only infected patients to IFN-α treatment and have better prognoses. These results suggest that the rate of HCV genotype 2 infection is higher in HCV patients co-infected with GBV-C/HGV than HCV-only infected patients. Moreover, in 2 of the 10 HCV and GBV-C/HGV co-infected patients, GBV-C/HGV was eradicated by IFN treatment but anti-E2 antibody did not develop in their sera (Table V), most likely because GBV-C/HGV was forcibly eradicated by the IFN.

In conclusion, the present study indicates that the rate of GBV-C/HGV co-infection of HCV carriers is 10% in Japan, and co-infected patients are likely to be younger and more sensitive to IFN-α treatment than HCV-only infected patients. In addition, anti-E2 antibody may be a neutralizing antibody for GBV-C/HGV.

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